ULK1 Kinase Assay

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Scientific Background:

ULK1 is a serine/threonine protein kinase that plays critical role during initial stages of autophagy which is a vital response to nutrient starvation. The conserved C-terminal domain (CTD) of ULK1 controls the regulatory function and localization of the protein. Knockdown of ULK1 inhibits the autophagic response as well as inhibiting rapamycin-induced autophagy consistent with a role downstream of mTOR (1). ULK1 forms a complex with FIP200 and ATG13 and this complex is essential for starvation-induced autophagy (2). Both FIP200 and ATG13 are critical for correct localization of ULK1 to the pre-autophagosome and stability of ULK1 protein. ULK1 is phosphorylated by the mTOR pathway in a nutrient starvation-regulated manner.

1. Ganley I G, et al: ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. J Biol Chem. 2009 May 1;284(18):12297-305.
2. Chan E Y, et al: siRNA screening of the kinome identifies ULK1 as a multidomain modulator of autophagy. J Biol Chem. 2007 Aug 31;282(35):25464-74.

ADP-Glo™ Kinase Assay

Description

ADP-Glo™ Kinase Assay is a luminescent kinase assay that measures ADP formed from a kinase reaction; ADP is converted into ATP, which is converted into light by Ultra-Glo™ Luciferase (Fig. 1). The luminescent signal positively correlates with ADP amount (Fig. 2) and kinase activity (Fig. 3A). The assay is well suited for measuring the effects chemical compounds have on the activity of a broad range of purified kinases—making it ideal for both primary screening as well as kinase selectivity profiling (Fig. 3B). The ADP-Glo™ Kinase Assay can be used to monitor the activity of virtually any ADP-generating enzyme (e.g., kinase or ATPase) using up to 1mM ATP.

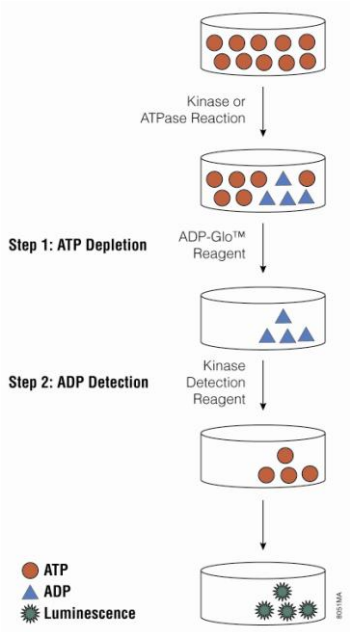

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

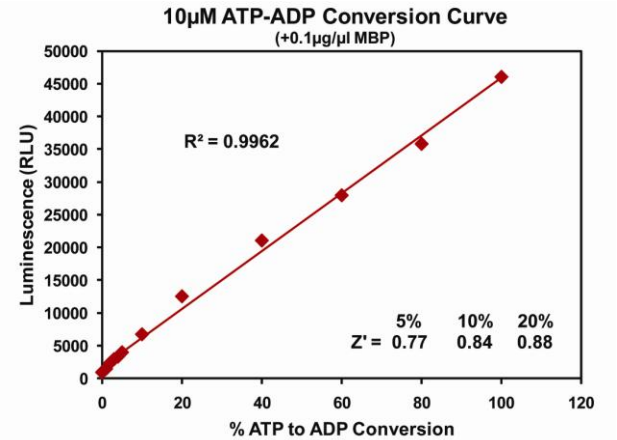

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase Buffer.
- Add to the wells of 384 low volume plate:
  - 1 µl of inhibitor or (5% DMSO)
  - 2 µl of enzyme (defined from table 1)
  - 2 µl of substrate/ATP mix
- Incubate at room temperature for 60 minutes.
- Add 5 µl of ADP-Glo™ Reagent
- Incubate at room temperature for 40 minutes.
- Add 10 µl of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1second).

Table 1. ULK1 Enzyme Titration. Data are shown as relative light units (RLU) that directly correlate to the amount of ADP produced. The correlation between the % of ATP converted to ADP and corresponding signal to background ratio is indicated for each kinase amount.

ULK1, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0
RLU	47106	40311	25047	13317	6963	4823	2832	2122	1509	835
S/B	56	48	30	16	8	6	3.4	2.5	1.8	1
% Conversion	99	84	51	25	11	7	2.3	1.8	1.2	0

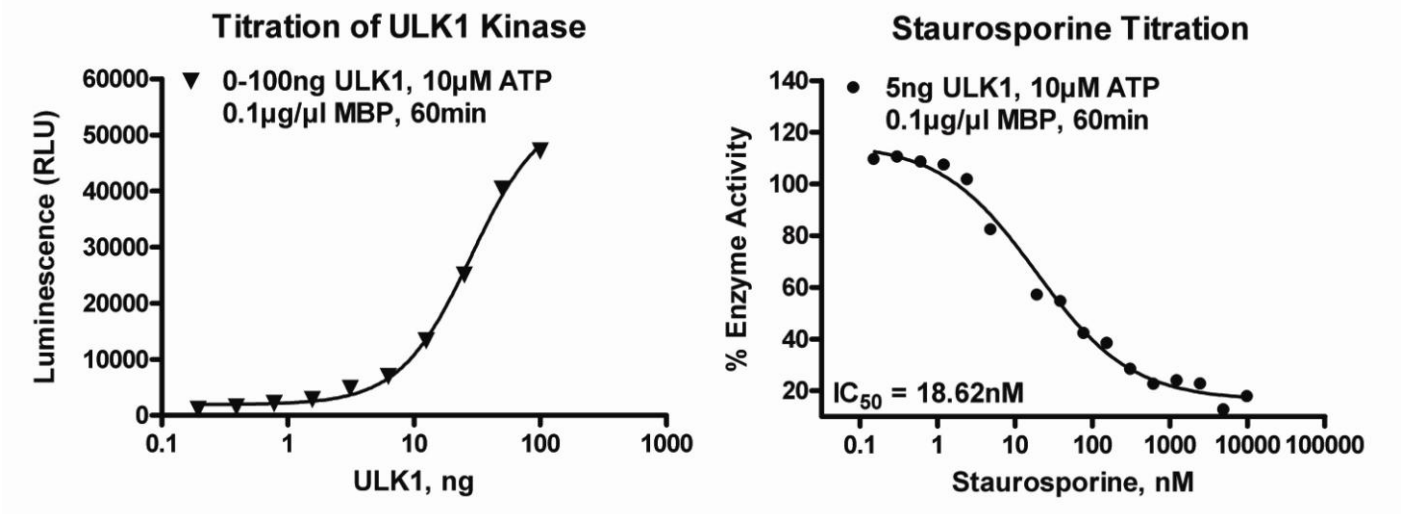

Figure 3. ULK1 Kinase Assay Development. (A) ULK1 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 5ng of ULK1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
ULK1 Kinase Enzyme System	Promega	V3521
ADP-Glo™ + ULK1 Kinase Enzyme System	Promega	V9191

ULK1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.